

## Comparisons of structural iron reduction in smectites by bacteria and dithionite: II. A variable-temperature Mössbauer spectroscopic study of Garfield nontronite\*

Fabiana R. Ribeiro<sup>1</sup>, José D. Fabris<sup>2</sup>, Joel E. Kostka<sup>3</sup>,  
Peter Komadel<sup>4</sup>, and Joseph W. Stucki<sup>1,‡</sup>

<sup>1</sup>Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; <sup>2</sup>Department of Chemistry, Federal University of Minas Gerais – Campus Pampulha, Belo Horizonte, MG, Brazil; <sup>3</sup>Department of Oceanography, Florida State University, Tallahassee, FL 32306, USA; <sup>4</sup>Institute of Inorganic Chemistry, Slovak Academy of Sciences, SK-845 36 Bratislava, Slovakia

**Abstract:** The reduction of structural Fe in smectite may be mediated either abiotically by reaction with chemical reducing agents or biotically by reaction with various bacterial species. The effects of abiotic reduction on clay surface chemistry are much better known than the effects of biotic reduction, and differences between them are still in need of investigation. The purpose of the present study was to compare the effects of dithionite (abiotic) and bacteria (biotic) reduction of structural Fe in nontronite on the clay structure as observed by variable-temperature Mössbauer spectroscopy. Biotic reduction was accomplished by incubating Na-saturated Garfield nontronite (sample API 33a) with *Shewanella oneidensis* strain MR-1 (Fe<sup>II</sup>/total Fe achieved was ~17 %). Partial abiotic reduction (Fe<sup>II</sup>/total Fe ~23 %) was achieved using pH-buffered sodium dithionite. The nontronite was also reduced abiotically to Fe<sup>II</sup>/total Fe ~96 %. Parallel samples were reoxidized by bubbling O<sub>2</sub> gas through the reduced suspensions at room temperature prior to Mössbauer analysis at 77 and 4 K. At 77 K, the reduction treatments all gave spectra composed of doublets for structural Fe<sup>II</sup> and Fe<sup>III</sup> in the nontronite. The spectra for reoxidized samples were largely restored to that of the unaltered sample, except for the sample reduced to 96 %. At 4 K, the spectrum for the 96 % reduced sample was highly complex and clearly reflected magnetic order in the sample. When partially reduced, the spectrum also exhibited magnetic order, but the features were completely different depending on whether reduced biotically or abiotically. The biotically reduced sample appeared to contain distinctly separate domains of Fe<sup>II</sup> and Fe<sup>III</sup> within the structure, whereas partial abiotic reduction produced a spectrum representative of Fe<sup>II</sup>–Fe<sup>III</sup> pairs as the dominant domain type. The 4 K spectra of the partially reduced, fully reoxidized samples were virtually the same as at 77 K, whereas reoxidation of the 96 % reduced sample produced a spectrum consisting of a magnetically ordered sextet with a minor contribution from a Fe<sup>II</sup> doublet, indicating significant structural alterations compared to the unaltered sample.

**Keywords:** redox; reduction; oxidation; cycles; nontronite; bacteria; shewanella.

\*Paper based on a presentation at the 8<sup>th</sup> Conference on Solid State Chemistry, 6–11 July 2008, Bratislava, Slovakia. Other presentations are published in this issue, pp. 1345–1534.

‡Corresponding author: Department of Natural Resources and Environmental Sciences, W-321 Turner Hall, 1102 South Goodwin Avenue, Urbana, IL 61801, USA; E-mail: jstucki@illinois.edu

## INTRODUCTION

The surface and colloidal behavior of clay minerals significantly influences many important processes occurring in soils, sediments, and industrial formulations. The oxidation state of iron (Fe) in the crystal structures of smectite clay minerals has been found to be a critically important factor underlying the behavior of the clay [1]. Changes in Fe oxidation state may occur either biotically due to the presence of Fe-reducing bacteria [2–5] or abiotically by reaction with inorganic reductants such as dithionite [6]. The link between clay behavior and Fe oxidation state was originally made from laboratory studies using chemical reductants. In natural systems, however, biotic pathways are the expected method for altering oxidation state in the clay. Akob et al. [7] and Stucki et al. [8] have, in fact, established that the addition of an electron donor to the soil or clay stimulates clay reduction and the thermodynamic order of electron acceptor utilization indicates that the reduction is microbial. The active Fe<sup>III</sup>-reducing bacteria have also been characterized [7]. Only a few studies, however, have investigated the similarities and differences between the effects of abiotic and biotic reduction on clay mineral properties and behavior.

In a companion study, Lee et al. [9] used infrared spectroscopy to compare the effects of biotic and chemical reduction pathways on the clay structure. Results revealed that: (1) the amount of structural Fe<sup>II</sup> that was produced by the biotic pathway was limited to less than approximately 1 mmol Fe<sup>II</sup>/g clay; (2) within this range of reduction, structural changes in the smectite were small compared to the dramatic changes observed by Fialips et al. [10,11] for abiotic reduction levels exceeding 3 mmol Fe<sup>II</sup>/g clay; (3) the relative effects of biotic and abiotic reduction were similar if the reduction level was similar; and (4) reoxidation generally restored the infrared spectrum of the smectite to its original appearance. These conclusions contradict previous assertions that bacterial reduction of structural Fe in smectites evokes extensive and irreversible changes in the clay structure [12–14]. Because the clay crystal structure is complex, consisting of many different types of chemical interactions, and infrared spectroscopy probes only the vibrational energies of interatomic bonds, further comparisons using complementary tools for structural analyses were warranted.

The significance of observation 2 above is that the relative fraction or percent of reduction in any given smectite will vary depending on the total Fe content. For Upton montmorillonite, which contains ~0.4 mmol total Fe/g, the fraction of reduction is virtually 100 %; whereas for Garfield nontronite, which contains ~4.5 mmol total Fe/g, the extent of reduction is only about 20 %. When comparisons are made, therefore, attention should be paid more to the absolute level of reduction (i.e., mmol Fe<sup>II</sup>/g clay) than to the percentage of total Fe reduced.

Because of its specificity for Fe, Mössbauer spectroscopy is uniquely suited to provide further structural information from redox-modified smectites, especially if employed over a wide range of temperatures (4–298 K). The Mössbauer spectrum of smectites is greatly influenced by the oxidation state of the Fe and by its spatial distribution and coordination environment throughout the clay lattice. Many studies have documented these parameters [1,15–20], namely, the isomer shift ( $\delta$ ), quadrupole splitting ( $\Delta$ ), and internal magnetic hyperfine field ( $B_{\text{HF}}$ ). The last of these occurs because of magnetic hyperfine interactions among the structural Fe cations of the clay mineral, which depend on the magnetic order due to structural Fe exchange interactions. Such interactions thus depend on the spatial distribution of the Fe. If, for example, the Fe is present in clusters, a significant domain of magnetically interacting ions will develop; whereas, if the Fe is dispersed among sites containing diamagnetic ions such as Al and Mg, no such magnetically ordered domains will exist. This case is possible only in the lower Fe-content clays. Fe-rich clays will unavoidably contain magnetic hyperfine interactions. The purpose of this study was to provide further comparative information regarding the changes in clay structure that occur due to biotic or abiotic reduction, as probed by variable-temperature Mössbauer spectroscopy.

## MATERIALS AND METHODS

### Clay preparation

Garfield nontronite, sample API 33a (Garfield, Washington, obtained from Ward's Natural Science Establishment), was used in this study. Its total Fe content is 24.15 wt % or 4.325 mmol/g clay. It was prepared in the same manner as described by Lee et al. [9]. Structural Fe reduction and reoxidation were also accomplished as described by Lee et al. [9], wherein the sample was reduced using either a pure culture of *Shewanella oneidensis* strain MR-1 [21] as described by Kostka et al. [22] or a pH-buffered solution of sodium dithionite [23]. The reduction level with dithionite was decreased to approximately that of the bacteria by restricting the reaction time with dithionite to 10 min. A second set of both bacteria- and dithionite-reduced samples was reoxidized after washing with 5 mM NaCl by bubbling O<sub>2</sub> gas through the reduced suspension for 24 h at room temperature. The actual levels of Fe reduction and reoxidation (i.e., Fe<sup>II</sup> contents) were obtained by chemical analysis using the 1,10-phenanthroline (phen) method [24,25]. Seven different treatments of the Garfield nontronite were examined, namely, the unaltered (UG), partially reduced by bacteria (BRG), partially reduced by dithionite (RG10), fully reduced by dithionite (RG), and reoxidized forms of the three reduced samples (BORG, ROG10, and ROG, respectively). Abbreviations for these samples are the same as given by Lee et al. [9].

### Mössbauer spectroscopy

Mössbauer spectra were acquired in transmission mode using a spectrometer supplied by Web Research Co., Inc., Edina, Minnesota, equipped with a Janis Model SHI-850-5 (Janis Research Co., Inc., Wilmington, Massachusetts) closed-cycle refrigerator (CCR) cryostat. This cryostat is capable of reaching temperatures in the region of 4.2 K without the need to add cryogenic liquids. Sample temperature was controlled with a Web model WTC102 auto-tuning temperature controller. Spectra were acquired in 1024 channels with a drive system operating with a triangular waveform. The source was nominally 50 mCi (1.85 GBq) <sup>57</sup>Co dispersed as 10 % in a Rh foil (Ritverc GmbH, St. Petersburg, Russia). A 7-μm thick, natural abundance Fe metal foil was used for velocity calibration of the spectrometer, and isomer shifts were expressed relative to the center of the Fe metal resonance.

The corresponding hyperfine parameters (Table 1) were deduced from fitting models for each spectrum based on paramagnetic doublets due to Fe<sup>II</sup> and Fe<sup>III</sup> in different chemical environments and sextets for Fe<sup>III</sup> when some magnetic ordering was evident. Fitting parameters for the spectra at 4 K for the abiotically partially and fully reduced samples and the biotically reduced sample, however, may not conform to any real physical model. The exception to this was in instances where a sextet was clearly evident in the overall pattern, in which cases arguments for physical attribution were made.

## RESULTS

The Mössbauer spectrum of smectites is greatly influenced by the oxidation state of the Fe and by its distribution and coordination environment throughout the clay lattice. The Mössbauer spectra were recorded at 77 and 4 K (Table 1). Consider first the spectra at 77 K. At this temperature, none of these samples contained a magnetically ordered component (sextet), except for a sextet with 2 % relative area in the unaltered sample (UG), which is attributed to a goethite impurity, similar to that observed by Murad [26] in ferruginous smectite. This phase was readily removed by the reduction processes and failed to reappear after reoxidation. The Mössbauer parameters for the fitted curves of the unaltered sample were consistent with only octahedral Fe<sup>III</sup>, which was almost equally distributed between an inner and an outer doublet (Table 1). On the extreme other end of this set was the fully abiotically reduced sample (RG) in which only octahedral Fe<sup>II</sup> was present in the Mössbauer spectrum (Fe<sup>II</sup>/total Fe confirmed by chemical analysis to be 96 % or >4.0 mmol Fe<sup>II</sup>/g clay) and the fitted subspectra were also almost equally distributed between an inner and an outer doublet. These two samples are regarded

Table 1 Mössbauer parameters<sup>a</sup> for Garfield nontronite at 77 and 4 K.

Temperature (K)		77	4										
Treatment	$\chi^2$	Site	$\delta$ (mm/s)	$\Delta$ (mm/s)	Width (mm/s)	BH <sub>f</sub> (T)	Area (%)	$\chi^2$ (mm/s)	$\delta$ (mm/s)	$\Delta$ (mm/s)	Width (T)	BH <sub>f</sub> (%)	Area
Unaltered	2.03	Fe <sup>3+</sup>	0.48(1)	0.23(1)	0.35(1)	–	55(2)	1.96	0.50(1)	0.23(1)	0.89(1)	–	83(2)
		Fe <sup>3+</sup>	0.47(1)	0.65(1)	0.33(1)	–	43(2)		0.46(1)	0.59(1)	0.40(1)	–	14(2)
Biologically reduced	1.41		0.49(1)	–0.24(1)	0.34(1)	50.0(1)	2(0)	1.65	0.41(1)	–0.36(1)	0.61(1)	49.8(1)	3(2)
		Fe <sup>3+</sup>	0.47(1)	0.39(1)	0.39(1)	–	63(3)		1.11(1)	3.54(1)	0.79(1)	–	4(1)
		Fe <sup>3+</sup>	0.47(1)	0.76(1)	0.29(1)	–	16(3)		0.91(2)	6.04(1)	2.7(4)	–	21(4)
		Fe <sup>2+</sup>	1.23(1)	2.69(1)	0.45(1)	–	21(0)		0.61(1)	1.48(1)	1.8(5)	–	34(2)
Abiotically partially reduced	4.43	Fe <sup>3+</sup>	0.43(1)	0.48(1)	0.43(1)	–	63(0)	1.53	0.51(1)	–0.06(1)	0.59(0)	47.1(1)	41(2)
		Fe <sup>3+</sup>	0.37(1)	0.96(1)	0.34(1)	–	18(0)		0.29(1)	0.82(1)	1.09(1)	–	30(2)
		Fe <sup>2+</sup>	1.44(1)	2.14(1)	0.31(1)	–	10(0)		1.57(1)	2.33(1)	1.11(1)	–	16(1)
		Fe <sup>2+</sup>	1.57(1)	2.31(1)	0.31(1)	–	9(0)		0.36(1)	–0.36(1)	5.12(1)	44.5(1)	54(4)
Abiotically fully reduced	5.34	Fe <sup>2+</sup>	1.27(1)	2.70(1)	0.45(1)	–	58(1)	1.64					
		Fe <sup>2+</sup>	1.27(1)	3.10(1)	0.31(1)	–	42(1)						
Biologically reduced reoxidized	2.25	Fe <sup>3+</sup>	0.47(1)	0.26(1)	0.39(1)	–	54(2)	1.29	2.73(1)	5.18(1)	0.94(1)	–	12(1)
		Fe <sup>3+</sup>	0.46(1)	0.68(1)	0.39(1)	–	46(2)		0.49(1)	0.18(1)	0.37(1)	–	14(8)
Abiotically partially reduced reoxidized	2.59	Fe <sup>3+</sup>	0.47(1)	0.23(1)	0.39(1)	–	53(3)	1.95	0.47(1)	0.57(1)	0.65(1)	–	86(8)
		Fe <sup>3+</sup>	0.47(1)	0.70(1)	0.43(1)	–	47(3)		0.48(1)	0.25(1)	0.45(1)	–	57(3)
Abiotically fully reduced reoxidized	1.55	Fe <sup>3+</sup>	0.47(1)	0.70(1)	0.42(1)	–	51(3)	1.72	0.47(1)	0.74(1)	0.47(1)	–	43(3)
		Fe <sup>3+</sup>	0.49(1)	1.15(1)	0.42(1)	–	40(3)		0.51(1)	–0.15(1)	2.11(1)	44.2(1)	93(2)
		Fe <sup>2+</sup>	1.15(1)	2.84(1)	0.35(1)	–	9(0)		0.52(1)	2.15(1)	0.71	–	7(1)

<sup>a</sup>Values in ( ) represent the uncertainty in the last digit.

as giving representative reference spectra for either a fully oxidized or a fully reduced structural Fe-rich nontronite, respectively.

Reoxidation of sample RG, giving sample ROG, restored much of the Fe<sup>III</sup> (Fe<sup>I</sup>/total Fe ~90 %), but even though the 77-K spectrum could be fitted with an inner and an outer doublet, the splittings were much greater than those found in the unaltered sample (UG). The electric field environment surrounding the Fe is, therefore, believed to be more distorted in the reoxidized nontronite than in the unaltered nontronite. This phenomenon has also been reported by others [27,28] for lower levels of reduction. The Fe<sup>II</sup> remaining in the RG sample after reoxidation was characterized by only one doublet with a quadrupole splitting between those of the two doublets used to fit the reduced spectrum. These results indicate that reoxidation failed to restore the abiotically fully reduced clay structure to its unaltered state. This structural perturbation is probably due to the irreversibility of Fe migrating from *cis*- to *trans*-octahedral sites during structural Fe reduction [29]. The extent of these distortions is revealed by the greater quadrupole splitting in the Mössbauer spectrum.

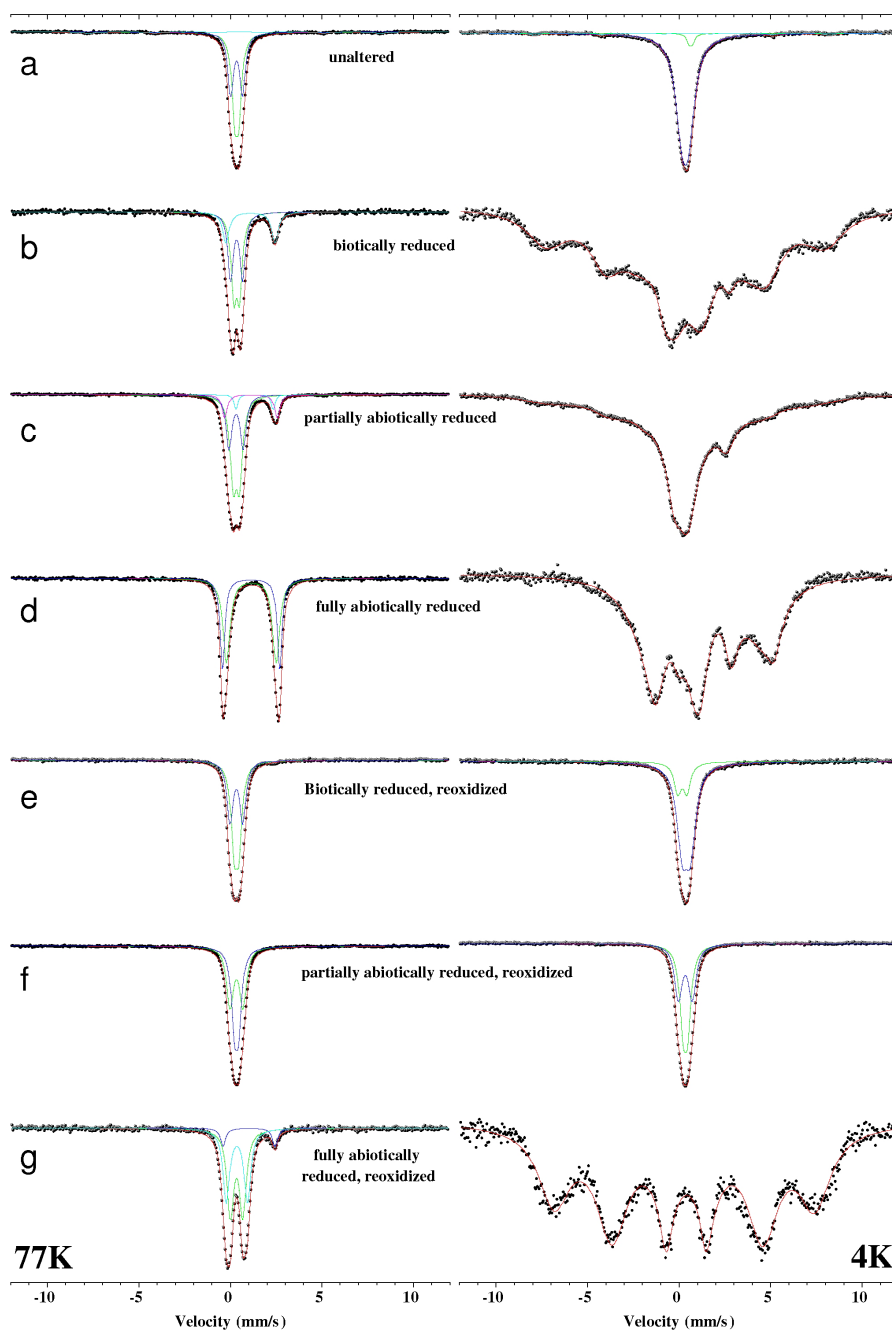
Partial reduction of Garfield nontronite (RG10) created a structure with a mixture of Fe<sup>II</sup> and Fe<sup>III</sup> in a ratio of about 1:4 (Fe<sup>II</sup>/total Fe = 23 %, ~1.0 mmol Fe<sup>II</sup>/g clay). The Mössbauer spectrum at 77 K was fitted with four distinguishable subspectra: two for Fe<sup>III</sup> and two for Fe<sup>II</sup>, distributed between inner and outer doublets. The splitting of both inner and outer Fe<sup>III</sup> doublets was greater than those found in the unaltered sample, and intensities of the inner and outer Fe<sup>II</sup> doublets were smaller than those found in sample RG (Table 1).

Biotic reduction of Garfield (BRG) also created a mixed-valent structure, with a Fe<sup>II</sup>/total Fe ratio of 17 % (~0.75 mmol Fe<sup>II</sup>/g clay), which is slightly less than the corresponding abiotic sample (RG10). At 77 K, the parameters for the inner and outer Fe<sup>III</sup> doublets resembled those of the unaltered sample. Only one Fe<sup>II</sup> doublet was resolved, however, and its quadrupole splitting was greater than either of those found in sample RG10, but was similar to that of the inner Fe<sup>II</sup> component found in sample RG. In both of the reducing processes, biotic and abiotic, more Fe<sup>III</sup> from the environment represented by the outer doublet was reduced than from the inner one.

The Mössbauer spectra and hyperfine parameters for reoxidized samples ROG10 and BORG resembled those of the unaltered sample (UG). Even the initial inner/outer doublet ratio was restored. In contrast, the Mössbauer spectrum of the fully reduced, reoxidized sample (ROG) was highly distorted and bore little similarity to the spectrum of the unaltered sample (UG) (Fig. 1). These observations led to the same conclusion as Lee et al. [9], as first postulated by Gates et al. [30], that reoxidation largely restores the clay structure to its original state, provided the initial level of reduction is modest (<1 mmol Fe<sup>II</sup>/g clay). They are also consistent with the results of Komadel et al. [31], who revealed by infrared spectroscopy and thermogravimetry that the structural OH content in abiotically highly reduced and reoxidized samples is about 15–20 % less than in the original nontronites, and of Fialips et al. [10,11], who showed by Fourier transform infrared (FTIR) significant structural alterations in reoxidized samples after high levels of reduction (>1 mmol Fe<sup>II</sup>/g clay).

Manceau et al. [29] studied the structure of reduced Garfield nontronite by several physicochemical methods. They suggested that some of the Fe atoms migrate from *cis*- to *trans*-sites in the reduced state, forming trioctahedral domains, separated by clusters of vacancies, within the structure of reduced nontronite. Migration of Fe from *cis*- to *trans*-sites during the reduction process was corroborated by simulations of X-ray diffraction patterns, which revealed that about 28 % of Fe<sup>II</sup> exists in *trans*-sites of the reduced nontronite, rather than fully *cis*-occupied, as in oxidized nontronite. This migration is accompanied by a dehydroxylation reaction due either to the protonation of OH groups initially coordinated to Fe and/or the coalescence of adjacent hydroxyls to form H<sub>2</sub>O as suggested by Stucki et al. [32] and Lear and Stucki [33]. This is in accord with the lower content of OH groups in reduced samples, which is not restored after reoxidation [31].

The Mössbauer spectra of the same set of samples at 4 K revealed extensive differences from the 77 K spectra because of the onset of magnetic order. These differences were least in the unaltered sample for which the spectra at 77 and 4 K still consisted of a central doublet and a small magnetically or-



**Fig. 1** Mössbauer spectra of unaltered (a), partially biotically reduced (b), partially abiotically reduced (c), fully abiotically reduced (d), and reoxidized forms of each reduced sample (e thru g, respectively) of Garfield nontronite at 77 K (left) and 4 K (right).

dered phase, except in the 4 K spectrum the central doublet was broadened, probably indicating the beginning of magnetic order among the  $\text{Fe}^{\text{III}}$  ions in the nontronite octahedral sheet. The similarity of the small magnetically ordered component in the unaltered sample at 77 and 4 K clearly demonstrated

that the Fe oxide impurity is not superparamagnetic and is assigned to goethite (see parameters in Table 1).

The fully abiotically reduced sample yielded a very unique spectrum which appeared to be magnetically ordered, but the pattern was unlike any other reported in the literature, with the overall features of the spectrum containing four broad peaks of different intensities. A theoretical model to fit the deconvolution of this spectrum has yet to be found but is the subject of ongoing further investigations. Because 96 % of the Fe in this sample is Fe<sup>II</sup>, the unfitted spectrum was adopted as the reference spectrum depicting an all-Fe<sup>II</sup> octahedral structure in nontronite at 4 K, even though the spectrum has yet to be fully interpreted.

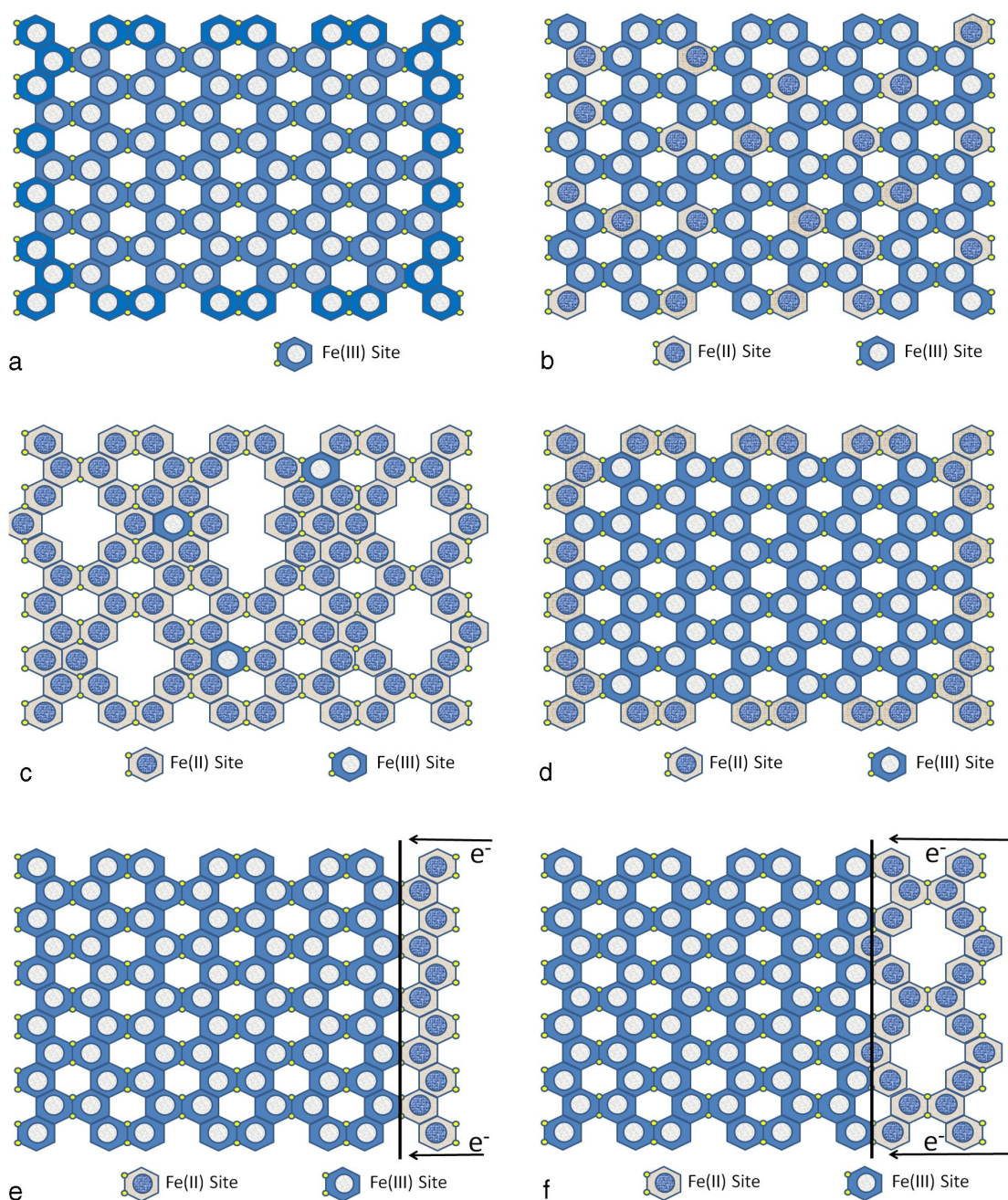
Partial reduction by biotic and abiotic methods reduced the nontronite to about the same Fe<sup>II</sup> content (17 and 23 %, respectively), but the respective Mössbauer spectra (Fig. 1) were dramatically different at 4 K, even though they were similar at 77 K. After abiotic reduction, the spectrum still exhibited a prominent central doublet for Fe<sup>III</sup>, but it was greatly flared at the base. A feature for a Fe<sup>II</sup> doublet was also visible at about 2.5 mm/s. Appearing out of the background of these Fe<sup>III</sup> and Fe<sup>II</sup> components was a sextet or sextets that produced broad but visible features at -7.5, -4.5, +5, and +8.5 mm/s (Fig. 1). The distance between the outer peaks of this sextet gave an estimated magnetic hyperfine field of 44.5 T (Table 1).

The spectrum from the biotically reduced sample (BRG) clearly differed from that of the partially abiotically reduced sample (RG10). Instead of having distinct features for Fe<sup>II</sup> and Fe<sup>III</sup>, it consisted of a set of peaks that clearly were similar to those observed in the spectrum for the totally abiotically reduced sample (RG), with one or more Fe<sup>III</sup> sextets superimposed. The estimated hyperfine field of the sextet was 47.1 T, which is characteristic of magnetically ordered octahedral Fe<sup>III</sup> in mixed-valent layer silicates [15,16,34]. This combination of peaks is interpreted here to indicate that the octahedral Fe is segregated into two domains; one that is primarily Fe<sup>II</sup>, which is responsible for the set of all-Fe<sup>II</sup> peaks, and one that is primarily Fe<sup>III</sup>, which accounts for the sextet(s). This follows the same reasoning as stated by Ballet and Coey [34] that “Magnetic ordering in iron-rich [layer-silicate] specimens at low temperatures results in spectra in which magnetic hyperfine structure from both the Fe<sup>2+</sup> and Fe<sup>3+</sup> components is resolved.”

## DISCUSSION

The exact pathway by which the electron enters the clay mineral structure to effect Fe reduction is still unknown, but results reported here give insight into the resulting distribution of Fe<sup>II</sup> following reduction and provide the basis for a more informed hypothesis. Lear and Stucki [35] reported that the Fe<sup>II</sup> distribution created by dithionite reduction is “pseudo random, with nearest neighbor exclusion,” meaning that the Fe<sup>II</sup> ions produced in the structure during partial reduction tend to be as far removed from each other as possible, thus creating Fe<sup>II</sup> ions surrounded by a Fe<sup>III</sup> matrix. This produces Fe<sup>II</sup>-Fe<sup>III</sup> rather than Fe<sup>II</sup>-Fe<sup>II</sup> pairs as the dominant Fe<sup>II</sup> domain type within the partially reduced structure (illustrated in Fig. 2b). This was confirmed by UV-vis spectroscopy, which monitored the Fe<sup>II</sup>-O-Fe<sup>III</sup> intervalence electron-transfer transition [35,36] and revealed that the number of Fe<sup>II</sup>-Fe<sup>III</sup> pairs increased almost linearly with Fe<sup>II</sup> content until a level of about 40–50 % reduction, then the intensity of this band decreased nonlinearly and disappeared upon complete reduction. Upon reoxidation, the intensity again increased to a maximum (presumably at about 50 % reoxidation), then decreased as oxidation neared completion. They reasoned that this scenario is evidence that the reduction is initiated at the basal surfaces of the clay layers rather than at the edges because the path length to Fe ions in the center of the layer is much shorter from the basal plane than from the edge of the layer.

The Mössbauer spectra (Fig. 1) for the case of partial abiotic reduction are consistent with these arguments and previous studies. While no clear evidence for a magnetically ordered Fe<sup>II</sup> domain was observed, some Fe<sup>III</sup> magnetic order was evident (broad features at -7.5, -4.5, +5, and +8.5 mm/s). The appearance of some magnetic order for Fe<sup>III</sup> under conditions of partial reduction was also observed by



**Fig. 2** Illustration of possible points of electron attack and resulting *cis*- vs. *trans*-octahedral site occupancies by  $\text{Fe}^{\text{III}}$  and  $\text{Fe}^{\text{II}}$  in unaltered (a), abiotically, partially reduced (b), abiotically fully reduced (c), and biotically reduced (d–f) nontronite. Patterns e and f illustrate the moving boundary of a reducing front, which could occur from one or more of the edges simultaneously (beginning with pattern d).

Lear and Stucki [37] and Schuette et al. [38]. Evidently, the introduction of  $\text{Fe}^{\text{II}}$  into the structure causes a shift in the magnetic exchange interactions within the lattice from frustrated anti-ferromagnetic to ferromagnetic.



The presence of larger, distinct domains of  $\text{Fe}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  within the clay after biotic reduction strongly suggests a different reduction pathway from the case of abiotic reduction discussed above. Instead of following the “pseudo random” model, biotic reduction follows a moving front model (Fig. 2c–f), as suggested by Komadel et al. [39] in which  $\text{Fe}^{\text{II}}$  is created initially at the edges of the clay layer, forming a thin  $\text{Fe}^{\text{II}}$  domain around the edge (Fig. 2c) with  $\text{Fe}^{\text{II}}\text{--Fe}^{\text{III}}$  pairs occurring only at the interface between the  $\text{Fe}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  domains. As the level of reduction increases, the interface between the  $\text{Fe}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  domains advances inward toward the center of the clay layer, causing the  $\text{Fe}^{\text{II}}$  domain to grow and the  $\text{Fe}^{\text{III}}$  domain to shrink (Figs. 2d–f). The features in the Mössbauer spectra revealing distinctly separate  $\text{Fe}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  domains are consistent with this scenario. This means that the point of contact between the clay and the bacterial cell or its electron shuttle is at the clay edges, not the basal surfaces.

Further evidence supporting the edge-contact model for biotic reduction is found in the fact that studies of bacterial Fe reduction in smectites have yet to achieve complete reduction of the structural Fe [2]. This result seems reasonable if reduction occurs from the clay edges, with the moving  $\text{Fe}^{\text{II}}\text{--Fe}^{\text{III}}$  boundary as described above. The total potential required to transport the electron the full distance from the layer’s edge to its center could be greater than provided by the bacterial reduction potential. This limitation would appear to be less if reduction were occurring from the basal surface. By these arguments, one is also led to state that dithionite reduction either occurs from the basal surfaces or the electrons produced by dithionite possess sufficient energy to create a conduction pathway from the edge to the center of the layer whereby the electron transfer to Fe can be induced at the nethermost points from the edges.

Reoxidation of the biotic and abiotic partially reduced samples yielded virtually the same Mössbauer spectra and parameters at 77 and 4 K. They also are similar to the unaltered sample. The parameter values for the outer quadrupole splitting in the abiotically reduced–reoxidized sample were slightly greater than those from the biotically reduced–reoxidized sample, at both temperatures, and also were greater than the parameters from the unaltered sample at the same temperatures. These differences could be due to variations in instrument performance between samples and/or to differences in sample preparation, but may also represent slight changes in the structure during the reduction and re-oxidation reactions.

The Mössbauer spectrum of fully abiotically reduced–reoxidized Garfield at 4 K exhibited extensive magnetic ordering of its components, but still contained a small amount (7 %) of residual  $\text{Fe}^{\text{II}}$ . The isomer shift attributed to this  $\text{Fe}^{\text{II}}$  is very low (0.52 mm/s) compared to the value of 1.15 mm/s at 77 K. This may mean that, simultaneous with the magnetic ordering, an electron delocalization occurs, spanning the entire crystallographic structure, which increases the ferric character of the high-spin ferrous Fe.

The mean magnetic hyperfine field is about 44.2 T, which is close to that observed in the abiotically partially reduced sample at the same temperature (44.5 T). This similarity in the magnetic field may be confirmation of the suggestions by Lear and Stucki [35] and Shuette et al. [36] that the presence of even a small amount of  $\text{Fe}^{\text{II}}$  in the structure changes the type of magnetic exchange interaction dramatically. Neither the field nor the shape of this spectrum resembles those of a  $\text{Fe}^{\text{III}}$  (oxyhydr)oxide, the presence of which in the abiotically reduced–reoxidized sample is improbable because reduction with sodium dithionite is well known for its ability to remove Fe oxides from soils and clays [40]. That this Fe is internal to the nontronite was confirmed by a further experiment (data not shown) in which the sample that was fully reduced, then reoxidized, was reduced again completely and reoxidized. The Mössbauer spectra obtained from these further reduction and reoxidation treatments gave the same patterns as those that have already been shown (Fig. 1). Therefore, even though the complete reduction–re-oxidation process appears to be irreversible with respect to the structure, the irreversibility clearly is not the result of formation of an external Fe (oxyhydr)oxide phase.

## ACKNOWLEDGMENTS

The authors are grateful for financial support of this study by the National Science Foundation, Division of Petrology and Geochemistry, Grant No. EAR 01-26308; and by the Environmental Remediation Science Program (ERSP), Biological and Environmental Research (BER), U.S. Department of Energy, Grant No. DE-FG02-07ER64374. Appreciation is also expressed to the U.S. Fulbright Scholars Program for supporting the scholarly exchanges of PK and JDF with the University of Illinois and to the Slovak Grant Agency VEGA, Grant No. 2/6177/27, for financial support of PK.

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